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INTERACTION OF 5-HYDROXYTRYPTAMINE AND d-LYSERGIC ACID DIETHYLAMIDE IN THE TRANSCALLOSAL RESPONSE

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IN an effort to study the effect of 5-hydroxytryptamine (5 HT) and d-lysergic acid diethylamide (LSD) in the CNS, Marrazzi and Hart (1,2) employed a modification of the method of Curtis and Bard (3) for transcallosally evoked cortical potentials. The former studies indicated that 5 HT inhibited the transcallosal response when injected intracarotidly. However, Koella and co-workers (4,5) found that the observed effect of intracarotidly injected 5 HT on potentials evoked by light flashes, is the algebraic sum of its effects at several sites, including carotid sinus, brain stem, and cortical receptors. In contrast, the experiments of Hart et al. (6) and Rodriguez et al. (7) demonstrated that inhibition of transcallosally evoked potentials can be obtained independently of effects on subcortical neurons or on baro- or chemoreceptors. Marrazzi and Hart (1,2) also showed that intracarotidly injected LSD inhibited the transcallosally evoked potential. The present investigation differed from previous studies in that experiments were specifically designed to determine whether 5 HT and LSD interact in the transcallosal preparation. Furthermore, the present experiments employed injection techniques designed to by-pass baro- and chemoreceptors. The construction of dose-transcallosal-inhibitory response curves for 5 HT and LSD was attempted as a means for studying the interaction of these agents. Unfortunately, the dosage sequences of 5 HT caused the

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appearance of tachyphylaxis while dosage sequences of LSD often produced what appeared to be cortical spreading depression. Hence, meaningful quantitative interaction relationships could not be derived. The tachyphylaxis to 5 HT was nonetheless turned to good cause as will be seen. Evidence is presented which demonstrated that these compounds are mutually antagonistic.

Methods

A series of 36 cats of either sex, weighing from 1.4 to 4.2 kg, were used in these experiments. Sodium pentobarbital anesthesia (35 mg/kg, intraperitoneally) was employed to prepare the animals as described by Marrazzi and Hart (1) for transcallosally evoked potentials. In 3 cats the cortex was partially deafferented as described by Grafstein (8). Three encéphale isolé animals were prepared under ether, then taken off anesthetic.

Non-polarizable Ag-AgCl recording electrodes were prepared after the manner of Rowland (9). Frontal bone was used as the reference point. This study is limited to potentials recorded from the region of maximum evoked response of the anterior suprasylvian gyrus. However, once this region was located, the stimulus strength (4 to 10 V) was adjusted midway between that which produced a minimal response and that which produced a maximal response.

The recording electrodes were connected in parallel to two channels of a Model 5 Grass Polygraph. One channel was DC coupled and served to indicate the occurrence of the phenomenon of spreading cortical depression (10).

The level of anesthesia (encéphale isolé preparations excepted) was controlled by pentobarbital, sufficient to abolish spontaneous electrical activity. Solutions of both 5 HT (as the creatinine sulfate complex) and LSD were freshly prepared in distilled water in a concentration of 500 µg/ml. Drugs were administered retrogradely via the cannulated lingual artery, ipsilateral to the recording electrodes, which permitted injection directly into the external carotid circulation thus, by-passing the carotid sinus. In addition to single injections, solutions of 5 HT were administered by perfusion, 0.04 to 0.1 ml/

min, with a Harvard Apparatus Company pump, Model #600-900. Solutions to be perfused were prepared so that 10 µg/kg 5 HT was contained in 0.04 ml.

Results

With respect to 5 HT injections, the encéphale isolé and the partially deafferented cortical preparations showed no qualitative differences from preparations deeply anesthetized with pentobarbital. The following results pertain specifically to the latter preparations.

Control of spontaneous activity decreased the variability of the evoked response to the extent that as little as 3 µg/kg 5 HT produced a noticeable decrease in amplitude (12 animals). Larger doses of 5 HT, up to 50 µg/kg, usually caused marked inhibition of the response. However, the sensitivity of the evoked potential to 5 HT varied from cat to cat. The degree of inhibition of the evoked potential caused by 5 HT in a given cat was dose-dependent; the change in the DC level did not appear to be dose-dependent in all animals. An example of these effects of 5 HT is shown in figure 1.

When the sequence of injections of 5 HT presented in figure 1 (3, 6, and 12.5 µg/kg) was repeated, 3 µg/kg caused no significant change in the amplitude of the evoked response. However, 6 and 12.5 µg/kg 5 HT were effective but the magnitude of depression was considerably less than that which resulted from the first series of injections. Various schedules of single doses of 5 HT were tried, but most animals became tachyphylactic to the effect of 5 HT on the evoked potential. This tachyphylaxis was not consistently observed in the response of the DC potential to 5 HT.

A decrease in amplitude or elimination of the evoked potential associated with a sudden shift in the DC level, indicative of the phenomenon of spreading depression (10), often occurred in these experiments. These findings underscore the necessity of monitoring the DC level when recording from the cortex.

Depression of the evoked potential by LSD was observed in doses as low as 6 µg/kg (16 animals). The magnitude of depression of the evoked potential

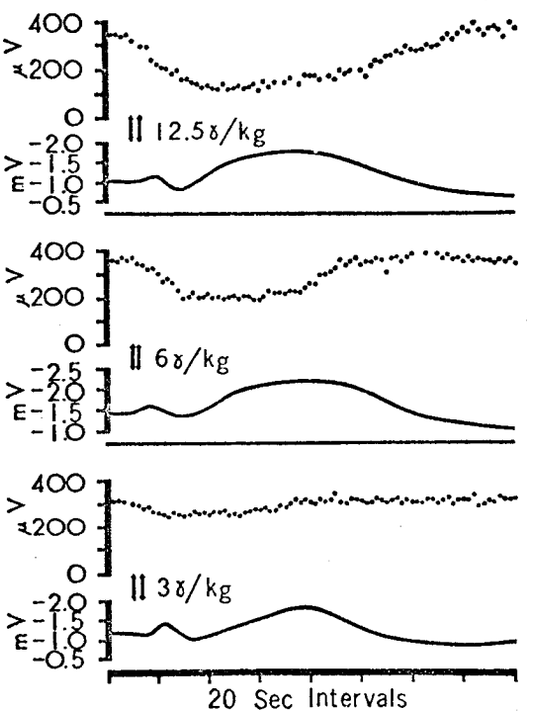

FIG. 1

Illustration of the effects of increasing doses of 5 HT on transcallosal and steady cortical potentials. 5 HT was injected at 15 min intervals beginning with 3 μg/kg. The first arrow designates injection of the drug; the second arrow represents rinse of the cannula with 0.2 ml saline. The dots represent peak amplitude of the negative wave of the transcallosal response in μV; the solid lines designate the relative DC level in mV. Time is represented in 20 sec intervals.

caused by a given dose of LSD, 6 to 100 µg/kg, varied from animal to animal.

In contrast to the negative shift of the DC level caused by 5 HT a positive shift resulted from LSD injection. An example of the effect of LSD on the evoked potential and on the DC level is shown in figure 2.

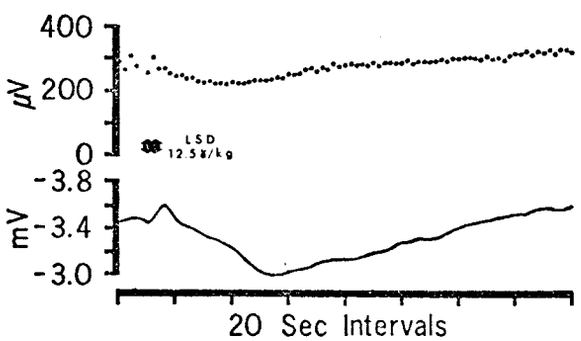

FIG. 2

Example of the effect of LSD on transcallosal and steady cortical potentials. Parameters and injection designations are the same as in Figure 1.

In order to determine a relationship between the dose of LSD and magnitude of depression of the evoked potential in a given animal, various doses of LSD were injected at 15 min intervals. In these instances, repeated doses of LSD, 6 to 100 µg/kg, eventually initiated cortical depression and associated DC shift. This phenomenon usually occurred when the total quantity of LSD administered reached approximately 100 µg/kg. On one occasion, five doses totaling 150 µg/kg were injected before this phenomenon took place.

Perfusion of 5 HT via the lingual artery was employed in an attempt to produce a constant depression of the evoked potential upon which the effect of a given dose of LSD could be examined. Prior to the start of 5 HT perfusion the response of the evoked potential to 6 µg/kg LSD was recorded.

Perfusion of 10 µg/kg/min 5 HT was found to depress the evoked potential (9 animals). However, the evoked potential was maintained at a reduced ampli-

tude (ca 50%) in only two of these animals. Figure 3 is an example of the latter result. The depressed potential in these two animals did not recover spontaneously. When LSD was injected the potential was momentarily abolished (fig. 3). Upon return, the potential steadily increased to approximately 75% of pre-perfusion amplitude. Additional LSD, injected in repeated doses up to 250 ug/kg, produced transient depression followed by gradual increase in amplitude of the potential but it never returned completely to pre-perfusion amplitude. Since the post-perfusion evoked potentials seemed not to return to baseline amplitudes spontaneously, but only heightened after LSD, it may be assumed that

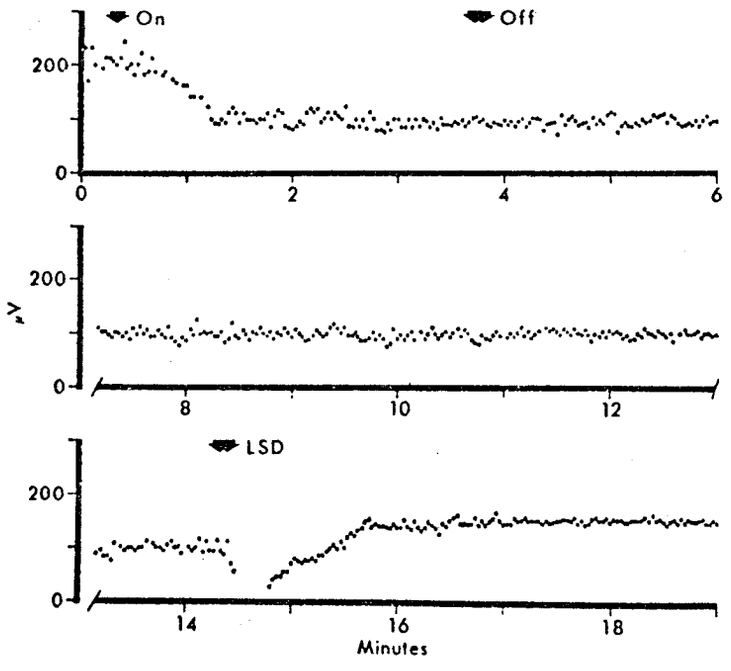

FIG. 3

Illustration of antagonism by LSD of the effect of 5 HT perfusion on the transcallosal response. 5 HT, 10 ug/kg/min, entered the animal at the arrow labelled "ON". The perfusion was terminated at about 4 min and the cannula was rinsed with saline. LSD, 50 ug/kg, was injected at about 14.5 min followed by rinse of the cannula.

the LSD antagonized the depressant effect of 5 HT.

Conversely, in the remaining 7 animals only an initial transient decrease in the amplitude of the evoked potential resulted from 5 HT perfusion (up to 5 min) demonstrating tachyphylaxis to the depressant effect of 5 HT on the evoked potential. The perfusion was stopped and repeated doses of 25 µg/kg LSD were injected until the evoked potential decreased markedly. The amplitude of the potential did not return spontaneously. Subsequent periods of 5 HT perfusion usually returned the LSD-depressed potential to pre-perfusion amplitude. Perfusions of saline were ineffective. The effectiveness of 5 HT perfusion diminished with the number of perfusion periods so that it was necessary to increase the perfusion rate in order to return the amplitude of the LSD-depressed potential to the pre-LSD level. When the perfusion, which was begun after LSD injection, was turned off, the potential amplitude fell again to low levels.

Eventually, perfusion of 5 HT, up to 50 µg/kg/min caused no further increase in amplitude of the evoked response. The degree of restoration of the evoked potential amplitude to control level varied from animal to animal. In 5 animals 5 HT perfusion returned the potential to control or greater than control amplitudes. In 2 cases, 5 HT perfusion eventually became ineffective and the post-LSD evoked potential disappeared.

A representative example of the latter interaction between 5 HT and LSD on the transcallosal response is presented in figure 4.

The DC potential, which otherwise remained relatively constant, shifted in response to perfusions of 5 HT and repeated doses of LSD. Both the direction and magnitude of the DC shifts varied considerably and were not consistently related to changes in amplitude of the evoked potential.

Discussion

The surface negative response of the cortex to callosal activation has been identified with the activity of apical dendrites (11). Barbiturate anesthesia has been reported to decrease spontaneous cortical activity and to de-

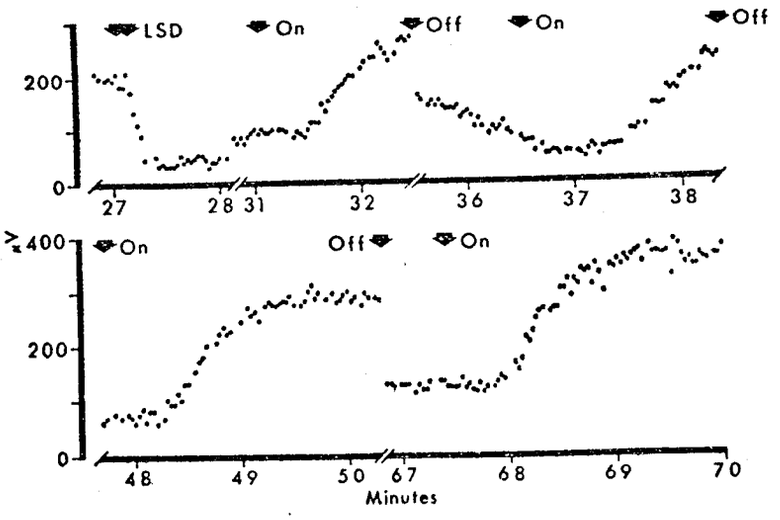

FIG. 4

Example of antagonism of the effect of LSD on the transcallosal response by intermittent perfusions of 5 HT. Periods of 5 HT perfusion, 10 μg/kg/min, are designated by the arrows labelled "ON" and "OFF". The amplitude of the potential decreased after 20 sec of 5 HT perfusion but returned to control level within 1 min even though the perfusion was continued indicating rapid onset of tachyphylaxis (not shown). Beginning at about 3 min, the perfusion of 5 HT was stopped, the cannula rinsed with saline, and 25 μg/kg LSD was injected at approximately 10 min intervals. This figure begins with the third dose of 25 μg/kg LSD followed immediately by 0.2 ml saline. The last perfusion period was terminated at 71 minutes (not shown) and the amplitude of the potential remained relatively constant (45 min) until termination of the experiment.

crease the variability in amplitude of evoked dentritic potentials (12,13). In the present investigation, deep barbiturate anesthesia also resulted in decreased variability of the evoked transcallosal dendritic response and thus provided a stable baseline for comparison of drug effects.

An advantage of the DC recording system was that the steady potential shift associated with cortical depression could be recorded. Potentials evoked by direct cortical stimulation, sensory reactions evoked by visual and somatic stimulation, and the transcallosal response have all been greatly reduced or abolished by the occurrence of cortical depression (10). The present results emphasize that unless the cortical steady potential is recorded simultaneously with evoked potentials, cortical depression may be confused with drug effects on the specific system being studied.

The occurrence of tachyphylaxis to the inhibitory effect of 5 HT, occurring during 5 HT perfusion, unmasked the antagonism of the inhibitory effect of LSD by 5 HT, otherwise, 5 HT and LSD might have acted additively. However, several periods of 5 HT perfusion were necessary to completely overcome the LSD effect. Between perfusion periods the amplitude of the potential decreased again. These effects suggest that 5 HT and LSD mutually displaced each other from receptor sites involved in depression of the transcallosal response. Support for an hypothesis of mutual displacement by these compounds comes from the work of Marchbanks, Rosenblatt, and O'Brien (14). This work demonstrated that binding of 5 HT to nerve-ending particles of the rat brain, particularly in the cortex, thalamus and midbrain, was inhibited by LSD. These workers also observed that binding of LSD to certain brain components was inhibited by 5 HT. Furthermore, studies of LSD antagonism of the hexobarbital potentiating effect of 5 HT led Brown (15) to suggest that LSD displaces 5 HT at receptor sites.

The results of the present investigation point out the complexities of efforts to analyze drug actions in the transcallosal preparation. Nevertheless, these results are the first relatively clear demonstration of mutual antagonism between LSD and 5 HT in a central nervous system pathway. The deep anesthesia utilized in these experiments sacrificed specificity and sensitivity necessary to determine the delicate quantitative relationships for the establishment of competitive antagonism. However, the results may reawaken interest in the hypothesis that the perversions of normal patterns of neural activity which result from LSD administration are due to an antagonism of endogenous 5 HT.

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